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Evaluation of vaginal culture results in patients with threatened preterm labor

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Abstract
Aim: In this study, we aimed to investigate the effects of certain infections on preterm birth by examining cervicovaginal cultures from patients with and without premature rupture of membranes (PROM) who were diagnosed with threatened preterm labor.
Material and Methods: Records of 85 patients diagnosed and treated for threatened preterm labor were retrospectively reviewed. Cervicovaginal cultures and laboratory results were analyzed. Preterm labor was diagnosed based on the Creasy-Herron criteria. Medical and obstetric histories, ultrasound examinations, vaginal examinations, and information on non-stress tests were recorded for all patients. The latency period was determined by subtracting the week of gestation at hospital admission from that at birth.
Results: A total of 74 patients who met the inclusion criteria were included in the study. Staphylococcus haemolyticus (n=7, 20.5%) was the most common microorganism detected in the cervicovaginal cultures. Births occurred before 37 weeks of gestation in 23 (67.6%) of the 34 patients who tested positive for microbial growth. No difference was observed between the rates of cervicovaginal culture positivity in threatened preterm labor patients with and without PROM (p=0.57). Of the 29 babies born in our hospital, thirteen (54.5%) of the 23 preterm babies were admitted to our hospital's neonatal intensive care unit. There were no positive indicators of infection or growth in the blood cultures of these infants.
Discussion: Cervicovaginal and urine cultures provide important information that may prevent severe mortality and morbidity during follow-up of patients with threatened preterm labor.

Keywords
Threatened Preterm Labor, Vaginal Culture, PROM

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Introduction

Infants born before 37 weeks of gestation are considered preterm [1]. The preterm birth rate in the United States is 10% [2]. Neonatal complications of preterm birth, associated with 70% of neonatal deaths, include respiratory distress syndrome (RDS), sepsis, intraventricular hemorrhage, necrotizing enterocolitis, hypothermia, hypoglycemia, hyperbilirubinemia, and nutritional disturbances [3,4]. Long-term conditions such as retinopathy of prematurity, cerebral palsy, and neurodevelopmental disorders have been previously studied [3]. Therefore, it is critical to identify, manage, and investigate the etiology of preterm births.

Causes of preterm births include infection, abruptio placentae, placenta previa, history of preterm births, pregnancies under 18 and over 40 years of age, malnutrition, low body mass index (BMI), fetal anomalies, fetal growth retardation, oligo-polyhydramnios, vaginal bleeding, PROM, etc. [5]. Knowledge of the etiology and appropriate interventions are essential to prevent perinatal mortality and morbidity. Forty to fifty percent of preterm births are infection-related [1,6]. Although infections via hematological procedures, invasive procedures have been previously described, infections via the ascending route are most common [1].

In this study, we aimed to investigate the effects of certain infections on preterm birth by examining the cervicovaginal cultures of patients with and without PROM who were diagnosed with threatened preterm labor.

Material and Methods

We retrospectively reviewed records of patients diagnosed and treated for threatened preterm labor with or without PROM between January 2019 and January 2021 at the Department of Obstetrics and Gynecology, XXX Hospital. Cervicovaginal cultures and laboratory results were obtained from 85 patients, 11 of whom were excluded from the study for various reasons, and the remaining 74 were analyzed for the study.

The inclusion criteria for the study were gestational age between 20 and 36 weeks and 6 days (gestational week confirmed by last menstrual period and first-trimester ultrasound) and a diagnosis of threatened preterm birth. The diagnosis of preterm labor was made based on the Creasy-Herron criteria that involved the detection of uterine contractions at a frequency of four per 20 min or eight per 60 min, and was accompanied by one of the following: PROM [detected by fluid leaking during speculum examination or by the test kit for the detection of alpha-microglobulin-1 in the placenta (Amnisure ROM test, N-Dia Inc, New York, USA)], cervical dilation exceeding 2 cm, effacement exceeding 50 %, or a change in cervical dilation or effacement detected by serial examinations [7]. Patients aged 18-40 years with no other known causes of infection were included. The exclusion criteria were age < 18 years, age > 40 years, and the presence of other known sources of infection. Patients with known conditions that may lead to preterm birth (e.g., uterine anomaly, fetal anomaly, cervical pathology, or fetal growth retardation), abnormalities of the placenta, oligo-polyhydramnios, vaginal bleeding, history of chronic concomitant diseases, smoking, alcohol, and substance use, and those who had taken antibiotics in the last 3 weeks were also excluded.

Medical and obstetric histories, ultrasound examinations, vaginal speculum and digital examinations, and information on non-stress tests were recorded for all the patients. The latency period was calculated using the following formula: gestational week at birth - gestational week at hospital admission.

Cervicovaginal culture results were recorded for all patients. At our hospital, vaginal culture specimens were delivered to the microbiology laboratory using Stuart Transport Swap and Medium (BTR-Gülkimya/Ankara or Fıratmed/Ankara). The samples were seeded on 5% blood agar and MacConkey agar, and the Candida positive samples were seeded on the Sabouraud medium. Microorganisms were identified by preparing fresh saline and performing Gram staining. The presence of trophozoites in fresh saline indicated the presence of Trichomonas vaginalis. Candida was identified based on the appearance of yeast, pseudohyphae and growth in culture. Bacterial vaginosis was diagnosed by Gram staining of coccobacilli with variable staining, detection of epithelial cells that are clue cells to which these bacteria adhere, and Nugent scoring.

This study was approved by the ethics committee of our hospital on October 7, 2020 (approval number: 2467).

Statistical Analysis

The SPSS 26.0 (IBM Corporation, Armonk, New York, United States) and MedCalc 14 (Acacialaan 22, B-8400 Ostend, Belgium) were used to analyze the variables. The Shapiro-Wilk-Francia test determined the fit of the univariate data to the normal distribution. The chi-square test was used to examine the correlations between the quantitative variables. Quantitative variables are reported as mean ± standard deviation (SD) and categorical variables as n (%). Variables were analyzed at a 95% confidence level, and p-values less than 0.05 were considered significant.

Results

A total of 74 patients who met the inclusion criteria were included in the study. Fifty-two patients (70.3%) were multiparous and 22 (29.7%) were primiparous. The ages of the patients ranged between 18-40 y (mean: 22.1 [SD:7.2] years). None of the patients were smokers or had any concomitant diseases. Growth was detected in cervicovaginal cultures from 34 patients (45%). Urine cultures in four patients showed growth, while seven patients (9.4%) presented with urine and cervicovaginal culture positivity simultaneously.

Staphylococcus haemolyticus (n=7, 20.5%) was the most common microorganism detected in the cervicovaginal cultures. Among the microorganisms grown in cervicovaginal cultures, the following types of micrroorganisms were detected: Staphylococcus haemolyticus (n=7), Candida tropicalis (n=4, 11%), Staphylococcus aureus (n=5, 14%), Escherichia coli (n=4, 11%), Staphylococcus intermedius (n=1, 2.9%), Candida albicans (n=3, 8.8%), Streptococcus agalactiae (n=1, 2.9%), Dermacoccus nishinomiyaensis (n=1, 2.9%), Staphylococcus hominis (n=1, 2.9%), Candida glabrata (n=1, 2.9%), Klebsiella pneumonia (n=1, 2.9%), Streptococcus pneumonia (n=1, 2.9%), Staphylococcus lentus (n=1, 2.9%), Pediococcus pentosaceus (n=1, 2.9%), Staphylococcus epidermidis (n=2, 5.8%). The

patients' demographic data are summarized in Table 1. Births occurred before 37 weeks of gestation in 23 (67.6%) of the 34 patients who tested positive for microbial growth. Preterm births were detected in six patients that presented with both urine and cervicovaginal culture growth. In four of these six patients, the same microorganisms were detected in both urine and cervicovaginal cultures (three Escherichia coli and one Pediococcus pentosaceus). Notably, the growth of microorganisms was different between the two patients (patient 1: vagina, Candida tropicalis – urine, Proteus mirabilis; patient 2: vagina; Klebsiella pneumonia – urine; methicillin-resistant Staphylococcus aureus).

In 29 (39.1%) patients, the indication for hospital admissions was threatened preterm labor that developed with or after the PROM, whereas 45 patients (60.9%) did not present with PROM. Of the patients with positive cervicovaginal cultures, 12 presented with PROM, and all delivered before 37 gestational weeks. No correlation was found between cervicovaginal culture positivity and patient age (P =0.85). Additionally, there was no correlation between cervicovaginal culture positivity and BMI (P=0.49). No difference was observed between the rates of cervicovaginal culture positivity in threatened preterm labor patients with and without PROM (p=0.57). There was no significant correlation between cervicovaginal culture positivity and time between liquid leakage and birth (p=0.75). In addition, no correlation was found between cervicovaginal culture positivity, WBC count and CRP levels at birth (p=0.54 and p=0.40, respectively).

Correlation analysis revealed a moderate positive correlation (p=0.04, r=0.24) between age and BMI, and a moderate negative correlation between age and gestational age at hospital admission (p=0.03, r=-0.25). A moderate positive correlation (p=0.04, r=0.35) was found between CRP level at hospital admission and BMI. A strong negative correlation was observed between the gestational week at hospital admission and latency period (p=0.00, r=-0.50). There was a positive correlation (p=0.00, r=0.58) between the latency period and the length of hospital stay. A strong negative correlation (p=0.00, r=-0.37) was also detected between the WBC count at hospital admission and latency period (Table 2).

Of the 74 pregnant women who were monitored for threatened preterm labor, 27 delivered at our hospital and multiple pregnancies were detected in two of these pregnant women. Of the 29 babies born in our hospital, six (20.7%) were term births and 23 (79.3%) were preterm births. The mean birth weight of the 29 babies was 2411.6±681 g, and the mean gestational week was 34.6±2.9. Ten preterm babies were born late preterm and discharged after follow-up with their mothers. Thirteen (54.5%) of the 23 preterm babies were admitted to our hospital's neonatal intensive care unit. The mean gestational week of the admitted babies was 32.5±2.5 (min-max, 27 GW -34 GW), and the mean birth weight was 1857.3± 498.4 g (min-max, 930 g-2580 g). The patients were hospitalized for premature births, respiratory distress, low birth weight, and sepsis. Four infants were mechanically ventilated for RDS and received intratracheal surfactant. Five infants required follow-up with noninvasive mechanical ventilation. There were no positive infection indicators or growth in the blood cultures of the infants admitted to the neonatal intensive care unit.

Table 1. Demographic and Biochemical Characteristics of the Patients with Threatened Preterm Labor +/- (Premature Membrane Rupture)

	Total (n=74) Mean (SD)
Age (year)	22.1 (7.2)
BMI (kg/m2)	27.78 (4.36)
Gestational week	31.58 (3.88)
Gestational week at birth	34.17 (2.85)
Latency*	11.1 (014.7)
CRP level (at birth)	41.85(58.57)
WBC count (at birth)	12.4 (4.62)

Latency, gestational week at birth–gestational week at hospital admission; BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell; SD, standard deviation

Discussion

This study aimed to investigate the rate of cervicovaginal culture positivity in patients admitted to our hospital with indications of threatened preterm labor with or without the PROM. In our study the cervicovaginal culture positivity was observed in 45% of the patients. Simultaneous urine and cervicovaginal culture positivity was detected in seven (9.4%) of these patients.

The relationship between the vaginal microbiota and preterm birth has been discussed in several studies, with the conclusion that vaginal infections can cause preterm birth [6,8,9]. Lockwood found that ascending genital infections can cause up to 50% of preterm births [6]. Klein et al. revealed that lower genital tract infections can induce preterm birth via cytokine production by leukocytes [9]. Miyoshi et al. found that a

Table 2. The correlation of clinical and laboratory data of patients with premature rupture of membranes and threatened preterm birth

	Age	BMI	Gestational week at hospital admission	Gestational week at birth	Latency	Length of hospital stay	WBC count at hospital admission	CRP level at hospital admission
Age	1	0.24* 0.04	-.25* 0.03	NS	NS	NS	NS	NS
BMI	0.24* 0.04	1	NS	NS	NS	NS	NS	0.35* 0.04
Gestational week at hospital admission	-.25* 0.03	NS	1	NS	-.50** 0.00	NS	NS	NS
Gestational week at birth	NS	NS	0.69** 0.00	1	NS	NS	NS	NS
Latency	NS	NS	-.50** 0.00	NS	1	0.58** 0.00	-.37** 0.00	NS
Length of hospital stay	NS	NS	-.27* 0.02	NS	0.58** 0.00	1	NS	NS
WBC count at hospital admission	NS	NS	NS	NS	-.37** 0.00	NS	1	NS
CRP level at hospital admission	NS	0.35* 0.04	NS	NS	NS	NS	NS	1

Latency: gestational week at birth–gestational week at hospital admission; BMI: body mass index; CRP: C-reactive protein; WBC: white blood cell; NS: non-significant
*The correlation is significant at the 0.05 level (2-tailed); **The correlation is significant at the 0.01 level (2-tailed).

positive vaginal Ureaplasma urealyticum/Mycoplasma hominis culture is a predictive factor for preterm births [10]. Arena et al. found that in women diagnosed with premature labor, all patients presented with vaginal dysbiosis [11]. Giraldo et al. found that microbiological examination of urine and genitalia identified an infectious agent in 49% of the cases [12]. The authors recommend early investigation and treatment of these infections.

Gardnerella vaginalis, Candida species, and Trichomonas vaginalis are the causative agents of vaginal infections [12,13]. Bacterial vaginosis increases the incidence of preterm birth two-fold [9]. Ten to fifteen percent of the women with bacterial vaginosis deliver prematurely [14]. Ugwumadu et al. revealed that the risk of preterm birth decreased when bacterial vaginosis was treated with oral clindamycin [15]. Interestingly, Gardnerella vaginalis was detected in only one patient in our study.

In a meta-analysis, Roberts et al. found that the treatment of pregnant women with asymptomatic vaginal candidiasis helped prevent preterm labor; however, further prospective studies are needed on this topic [16]. In contrast, Schuster et al. reported that treatment with asymptomatic vaginal candidiasis had no effect on preventing preterm births [17]. Although there are differing results in the literature, vaginal Candida tends to not affect preterm birth [9]. In our study, Candida species were detected in seven patients (9.4%), four of whom delivered before 37 weeks of gestation.

Trichomonas vaginalis infection is associated with complications such as PROM, preterm birth, low birth weight [12]. Interestingly, treatment of patients with asymptomatic Trichomonas was associated with an increased risk of preterm birth; it was suggested to avoid treatment of these patients [18]. Inflammatory mediators released by deceased Trichomonas might contribute to the increased risk of preterm birth [18], and treatment is recommended for symptomatic Trichomonas vaginitis [9]. Trichomonas was not detected in our patient group. In recent years, the incidence of Trichomonas in the general population may be decreased, because of timely screening and treatment.

GBS was detected in one of the enrolled patients. Since these bacteria do not cause preterm birth but can cause severe neonatal complications, they must be treated immediately after detection [9]. A 2017 meta-analysis pointed out that maternal GBS colonization may be associated with preterm birth and emphasized that this association was stronger when colonization was detected in urine [19]. In contrast, our patient had a preterm birth at 34 weeks and received prophylaxis against GBS during birth.

Culture positivity was detected in simultaneous vaginal and urine samples from seven of our patients, six of whom delivered preterm babies. Of the three patients in whom culture positivity was detected only in the urine sample, two had preterm births. Since tract infections in pregnancy can lead to severe morbidity and mortality, immediate treatment is recommended after detection [20]. If asymptomatic bacteriuria is not treated, an estimated 30% of the cases develop acute pyelonephritis, which can lead to complications such as preeclampsia, preterm birth, and low birth weight [20].

Consistent with the few studies published in the literature, we found no association between the latency period and culture positivity in our study [21]. Rouzaire et al. in their study [21] noted a vaginal culture positivity in 28.2% of the patients with PROM and noted that culture positivity had no effect on the birth time. In a large-scale retrospective study, Dagklis et al. reported that clinical chorioamnionitis reduced the latency period. However, data on culture positivity in these patients were not shared [22]. We also found no statistically significant differences in WBC counts and CRP levels at birth between the culture-positive and culture-negative patients. The time between membrane rupture and birth in patients with PROM did not vary between the culture-positive and culture-negative patients. There was no difference in culture positivity between patients with PROM and those with spontaneous preterm births. The observed decrease in the latency period with increasing gestational age is consistent with previous reports [22,23]. The negative correlation between the WBC count at hospital admission and latency is consistent with the view that clinical chorioamnionitis shortens the latency period [23].

Considering the 45% cervicovaginal culture positivity in our study group and the morbidity and mortality caused by infections during pregnancy, the diagnosis and treatment of these infections in pregnant women are important. Tract infections have similar implications.

Additionally, newborns of pregnant women monitored for threatened preterm labor were evaluated for sepsis. None of the patients had sepsis, and the acute-phase reactants tested negative. PROM has been reported to be a risk factor for the development of neonatal sepsis [24]. The incidence of neonatal sepsis in pregnant women with PROM varies from 1 to 13.01%, depending on the duration of membrane rupture [24,25]. Ocviyanti et al. reported that the risk of sepsis increased 18.59-fold in preterm babies born to mothers with PROM compared to full term babies [25]. The authors noted that the group at the highest risk was preterm babies with a gestation period of less than 28 weeks [25]. In their study, only one case of neonatal sepsis was identified in 405 term infants born after PROM. The fact that no newborns developed sepsis in our study suggests that this is related to maternal antibiotic treatment, a smaller number of patients, and at higher gestational age. This is one of the rare studies evaluating vaginal culture outcomes in women with threatened preterm labor and the neonatal outcomes of these pregnancies.

Our study has some limitations. This was a single-center, retrospective study with a relatively small number of patients. Larger prospective studies may yield more significant results.

Conclusion

Cervicovaginal and urine cultures provide important information that may prevent severe mortality and morbidity during follow-ups of patients with threatened preterm labor. Accumulating data on this topic may provide a better understanding of this clinical condition.

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Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content

including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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